THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 69:215-218, 1977

Vol. 69, No. 2
Printed in U.S.A.

THE INDUCTION OF IMMUNE TOLERANCE IN DELAYED CONTACT SENSITIVITY BY THE USE OF CHEMICALLY RELATED SUBSTANCES OF LOW IMMUNOGENICITY

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Immune tolerance in delayed contact sensitivity to pentadecylcatechol can be induced by a series of derivatives substituted in the 6 position of the ring. Some of these derivatives have the property of being very poor sensitizers and having very low dermal toxicity. Thus, sensitization and tolerance have different biologic mechanisms and are associated with different properties of these chemicals.

Contact dermatitis is a widespread problem that results from contact with industrial, experimentally prepared, or naturally occurring chemicals either where they are manufactured, where plants grow, or among those who use or consume such substances. Poison ivy or oak contact dermatitis is an important example. Once sensitization is established, it is difficult to eliminate; therefore, the simplest solution is removal of the offending substance from the environment. If contact with the sensitizer cannot be avoided, consideration may be given to the induction of immune tolerance or nonreactivity prior to the first contact. In this way, severe dermatitis may be avoided; this approach might prove to be valuable for poison ivy and poison oak.

Immune tolerance in delayed contact sensitivity can be induced in various ways. If the route of administration does not lead to sensitization, it usually results in tolerance. Thus, intravenous injection [1], oral administration [2,3], subcutaneous injection without adjuvant [3], or dermal application [4-7] may, with selected chemicals, result in tolerance. Another approach is the use of a modified related substance, i.e., a poor, or a nonsensitizer, to induce tolerance. Thus, dinitrobenzesulfonate [8] and dichloronitrobenzene [9] induce tolerance to dinitrofluorobenzene.

The simplest procedure for inducing immune tolerance to poison ivy-poison oak would be to inject doses of the active principle dissolved in oil. The active principle of poison ivy and poison oak can be extracted from leaves and stems of the plants and is a mixture of pentadecyl and heptadecyl catechols, usually referred to as urushiol. This procedure would be analogous to the induction of immune tolerance using pure pentadecylcatechol itself [3]. There are two disadvantages to the use of urushiol: (1) it is a strong primary irritant which limits the size of the dose and the method of administration, and (2) it is a potent sensitizer so that, if not administered with great care, the result may be sensitization rather than tolerance.

In earlier investigations on pentadecylcatechols, we found that derivatives resulting from alterations in the ring OH groups were nonsensitizing and induced either little or no detectable tolerance [10]. The current study describes a series of derivatives with varying sensitizing capacity and primary irritancy that induce intense tolerance to the potent sensitizer 3-*N*-pentadecylcatechol (PDC).

MATERIALS AND METHODS

The chemicals employed were all substituted in the 6 position of the benzene ring of PDC (Fig. 1). Their structure and synthesis are described elsewhere [11]. The animals were approximately 350-gm, male, Hartley-strain guinea pigs obtained from the Animal Production Section of the NIH. Upon receipt, they were randomly assigned to cages employing a table of random numbers. Animals were sensitized (Tab. I) by injecting each compound emulsified in complete Freund's adjuvant on successive weeks into the nuchal area, inguinal-axillary areas, and the footpads; a total of 1 ml containing 1 mg of each substance was used. Increasing the dose to 10 mg did not lead to increased sensitivity. Tolerance (Tab. II) was induced by injecting a total of 2 mg of the chemical, in 1 ml of the mineral oil Drakeol 6VR (Penreco Inc., Butler, Pa.) subcutaneously on successive weeks into the nuchal area, inguinal-axillary areas, and the footpads; controls received only oil. Two to three weeks later all animals received 1 mg of PDC in 1 ml of complete Freund's adjuvant [12]. Three weeks later they were skin tested with varying doses of PDC, and then every second week for a total of three skin tests. Since animals increase in sensitivity for the first few weeks, their sensitivity during this time is not stable, and results of the first two skin tests are not useful for quantitatively expressing the degree of sensitivity as geometric means [12-14]. The third skin test results, therefore, are recorded. Skin testing was carried out by dropping 5 μl of serial 2-fold dilutions of PDC in acetone onto the clipped skin of

Manuscript received January 28, 1977; accepted for publication March 17, 1977.

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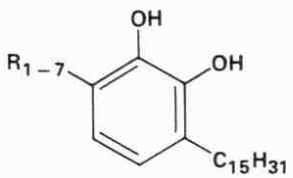
Abbreviations:
PDC: 3-*N*-pentadecylcatechol

a guinea pig. The solutions were dropped inside a brass ring of 7-mm diameter, thus giving reactions of approximately equal diameter. The lowest concentration of PDC giving observable erythema at 48 hr was recorded.

In order to calculate geometric mean values, nonreacting animals were assigned a value of 5 times the maximum test dose used for that substance. The maximum test dose was estimated [15] from the threshold inflammatory dose on normal guinea pigs. From the number of individuals reacting at each dose the geometric mean reactive dose was calculated (the last column in Tabs. I and II). The data in the Tables represent the sum of all animals used in 1-3 trials with each compound. Because of this, and because there were a few unrelated deaths among animals, the number of animals in each group is not identical.



R_1 = Diethylaminomethyl (DeaM) $-CH_2-N-(C_2H_5)_2$

R_2 = Carboxy (Carb) $-COOH$

R_3 = Morpholinomethyl (MoM) $-CH_2-N$ O

R_4 = Pyrrolidinomethyl (PyrM) $-CH_2-N$

R_5 = Carboethoxy (Carboeth) $-COOC_2H_5$

R_6 = Dimethylaminomethyl (DmaM) $-CH_2-N-(CH_3)_2$

R_7 = Piperidinomethyl (PipM) $-CH_2-N$

FIG. The formulae of the 7-substituted pentadecylcatechols.

RESULTS

The 6-substituted PDC compounds differed from PDC in two respects. They had markedly less primary irritant effect so that the guinea pigs could be tested at a maximum of 50 nmole, compared to 10 nmole for PDC. Secondly, they were relatively poor sensitizers. Thus, very few animals reacted to less than 1 nmole (Tab. I) with geometric mean reactivity having a range of 7.9 to 226; many animals failed to react at the highest test dose. As part of the third skin test with the homologous sensitizing compound, the animals were simultaneously tested on the opposite flank with PDC to determine whether their low reactivity was due to a poor eliciting compound [13]. The animals were somewhat less reactive to PDC than to the homologous compound. For comparison, most animals sensitized to PDC became sensitive and were much more reactive, having geometric mean reactivities of 0.7 (Tab. II, *oil control*), 2.1 [10], and 2.9 [16] nmole.

When tested for their ability to induce tolerance to the related PDC, these same substances were found to induce varying degrees of tolerance (Tab. II). A significant number of animals pretreated with one of the 6-substituted PDCs failed to react at the highest test dose of PDC, or reacted mainly at the highest test dose. For example, 15 of 16 animals pretreated with PipM (Tab. I) could not be sensitized to PDC. Thus, pretreating with related chemicals of low primary irritancy and poor sensitizing ability could reduce or prevent sensitization to the potent sensitizer PDC.

DISCUSSION

The use of chemically related substances of low or no detectable sensitizing capacity to induce immune tolerance to a potent sensitizer is an important finding.

There is much discussion concerning the mechanism of the induction of tolerance to sensitization to simple chemicals. Some studies suggest dissem-

TABLE I. *Skin reactivity of guinea pigs sensitized to various catechols substituted in the 6 position*

Sensitizing and test substance	Skin test dose (*nmole*)									Geom. mean[b]
	NR[a]	50	25	10	5	2.5	1.25	0.625	0.3125	
DEAM[c]	1[d]	0	0	5	1	0	0	1		7.9
Carboxy	5	0	0	0	1	0	1	0	1	12.5
MoM	4	5		1	3					38.5
PyrM	4	6	2	2	1	1				40.2
Carboeth	4	2	1	1						115.3
DmaM	4	2								144.0
PipM	15	1								226.0

[a] NR = Animals that failed to react at the highest test dose.

[b] To calculate the geometric mean, the nonreactors are assumed to react at 5 times the maximum test dose.

[c] For explanation of symbols see the Figure.

[d] The numbers represent the number of animals reacting at this test dose, but at no smaller test dose.

TABLE II. *Induction of tolerance to PDC by various 6-substituted catechols*

Injected substance	Skin test dose of PDC (*nmole*)							Geom. mean[b]
	NR[a]	10	5	2.5	1.25	0.625	0.3125	
DEAM[c]	0[d]	6						10.0
Carboxy	2	6						15.0
MoM	6	5	4	0	0	0	1	12.4
PyrM	4	8	1	1	2			10.1
Carboeth	0	1	6	0	1	2	1	1.9
DmaM	1	11	1	1	1			6.9
PipM	3	9	3	1				10.9
Oil (control)	0	2	0	0	3	1	2	0.7

[a] NR = Animals that failed to react at the highest test dose.

[b] To calculate the geometric mean, the nonreactors are assumed to react at 5 times the maximum test dose.

[c] For explanation of symbols see the Figure.

[d] The numbers represent the number of animals reacting at this test dose, but at no smaller test dose.

ination of the chemical through the blood [5], but other data demonstrate that a direct route between the site of administration and the draining lymph node may be critical [6,17]. The ability of certain substances to induce tolerance without much sensitivity is further indication of a difference in the mechanism of the two processes. Although tolerance and sensitization are competitive and simultaneous processes [18,19], the degree of sensitivity is determined by some still unknown mechanism. Therefore, it could appear that the chemical reactivity of the substance as well as the route must exert a role. In this study, all of the substances were administered by the same route; therefore, differences must be related to either physical or chemical properties. Differences that could correlate with their immunologic properties are not obvious at this time. It has been suggested that the proliferation or activation of suppressor cells may be important in the induction of tolerance in delayed sensitivity [19]. The cells involved may be B cells [20].

These data demonstrate that the toleragenic properties of some catechols related to PDC are separate from their sensitizing properties. However, catechols previously studied that were shown to be complete nonsensitizers were nontoleragenic [10]. Although it has been claimed that a nonsensitizing substance can induce tolerance to a chemically related sensitizer [9; see also 21 for a review], the data in this paper, together with that previously obtained [10], do not unequivocally support this concept. Perhaps studies with additional compounds related to PDC may help to clarify this problem.

This work may have clinical usefulness. It is a common practice to attempt to hyposensitize poison ivy- or poison oak-sensitive individuals by the administration of urushiol (a mixture of pentadecyl and heptadecylcatechols) [22], or plant extracts containing this material [23]. However, hyposensitization is difficult to achieve, is not regularly effective, and may be only temporary [23]. Consequently, it may be advantageous to induce immune tolerance in nonsensitive individuals at high risk of sensitization either because of their occupation or geographical location. Substances of the type described in this paper are of low toxicity and immunogenicity and may prove to be useful candidates for such purposes.

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